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DEPARTMENT OF THE ARMY
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#998

AN EVALUATION OF VACCINES AND THE EFFECTIVENESS OF VACCINATIONS AGAINST TYPHOID FEVER

[Following is a translation of an article by Janina Plachcinska in the Polish-language periodical Przeglad Epidemiologiczny (Epidemiologic Review), Vol 17, 1963, pages 67-69.]

X. Test of Passive Resistance on Chick Embryos With Serums of Persons Immunized with N, P, S and T Vaccines

(From the Epidemiology Plant of the Medical Academy in Warsaw and the Epidemiology Plant of the State Hygiene Establishment)

Before proceeding to make controlled vaccinations in 25 districts, initial tests were conducted to check post-vaccination reactions and evaluate the immunizing power of N, P, S and T vaccines by serologic methods in persons immunized with these vaccines. The mode and conditions of vaccination and blood sampling have been discussed in the paper by Zolnierkowa and Przestalska (7). The results of the reactions of agglutination and passive hemagglutination were given in the paper by Kopacka and Slubicka (4).

The purpose of our work was to determine, in the serums used before and after vaccination, the level of protecting antibodies determined by the Grabar test, for passive resistance on chick embryos (2), and, on this basis, to compare the immunogenic potency of N, P, S and T vaccines.

Materials and Methods

Serums. From blood taken before vaccination and two weeks after the second vaccination, serum was extracted in the Wojewodzstwo Epid. San. Station in Wroclaw and sent to the State Hygiene Establishment, where, after being put into ampules, it was kept at -20°C. The serums were furnished to the laboratories without giving their symbols, so that their potency was determined by a passive test without knowing for which vaccine they were used. The serums were defrosted in a +4° cooler and sterility was confirmed by transplants. Testing was done always in pairs, i.e., serums originating from the same person -- before and after vaccination. Diluting was done with a physiological NaCl solution in the proportion of 1:10 and the batches thus diluted were added to an equal volume of infecting emulsion immediately before infecting the embryos.

Strains. Strain S. typi Ty 2, obtained from the Pasteur Institute in Paris, was used. The strain was kept on flaked (klutym) agar; it was lyophilized in 1958 and again lyophilized from that revived on 25 August 1961, in an amount sufficient for all the experiments (60 ampules). The infecting emulsion was prepared, determined and checked in the manner discussed in the paper on the standardization of the active test on mice (5).

Chick Embryos. Ten-day chick embryos were used for the tests. A 0.2 ml mixture of serum with infecting emulsion or 0.1 ml of the infecting emulsion itself was injected into the urethral membrane or the urethral orifice for the control determination of LD 50. A constant dose of serum and variable infecting doses were used, at least five embryos being infected with each dilution of emulsion mixed with the constant dose of serum.

Course of Results of the Experiments

The LD 50 of the infecting emulsion for chick embryos was determined in several initial experiments. Then, on the basis of Grabar's studies (2,3) and our own observations (6), the infecting doses were established for embryos protected by the serum under investigation. Finally, infecting doses of 3, 9 and 27 bacteria were established for the serum used before vaccination and doses of 9, 27, 81, and 243 bacteria for the serums used after vaccination. At the same time a simplification was introduced, consisting in administering the material under

inv...tration, not in the urethral membrane, as was done heretofore, but in the urethral orifice.

The results of the experiments were read off on the third day, the number of dead embryos being determined. Then the LD 50 was computed by the graphic method (1), and the statistical reality of the difference between each two numerical values obtained was determined.

Among the 51 pairs of serums investigated, 16 came from persons immunized with vaccine N, 14 with vaccine S, 12 with vaccine P, and 9 with vaccine T.

Table I shows the ratio of the number of serums protecting in a statistically significant manner to the total number of the serums investigated in a given group. A compilation is given separately for each vaccine.

Table I

Protective Properties of Serums of Persons Vaccinated against Typhoid Fever

	1) Rodzaj szczepionki			
	N	P	S	T
2) l. surowic chroniących / 3) l. surowic badanych	7/16	6/12	2/14	1/9
4) % surowic chroniących	44	50	14	12

Legend: 1) Kind of Vaccine
2) Number of protecting vaccines
3) Number of serums investigated
4) Percent of protecting vaccines

Discussion and Conclusions

The purpose of the tests, as stated in the introduction, was to ascertain the growth of protective antibodies in the serum of persons vaccinated with various serums. The level of these antibodies can be determined on mice or chick embryos. Two considerations favored the choice of embryos: the much smaller amount of serum needed to make this test, and the greater objectivity of the test, resulting from the total humoral neutrality of the embryo itself. This test was simplified in the course of the investigation by changing the manner of introducing the mixture of serum

ther investigation with the bacterial emulsion, which is easier in the infecting technique and facilitates the reading of the results, since the displacement of the embryo with the formation of an air chamber is avoided.

Our observations must be regarded as tenative in view of the quite small number of persons tested with the several vaccines and the change in tecnique introduced during the tests. The results obtained do, however, permit us to draw the general conclusion that the method under discussion is sufficiently sensitive to enable one to distinguish the action of the various vaccines. As a result of the tests, it was possible to divide the vaccines into two groups: to the first belong vaccines P and N, to the second S and T, with no considerable distinction between the members of a pair. The serums obtained after immunization with vaccines P and N protected in 50 and 44%, but after immunization with vaccines S and T, in 14 and 12% [of the cases?].

The LD 50 of the serums taken before vaccination of the persons always formed on the level of the control LD 50, or else the serum of the nonimmunized persons did not contain natural protecting antibodies.

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